

All plasma products are not created equal: Characterizing differences between plasma products

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BACKGROUND: Plasma can be manufactured by multiple methods. Few studies have compared quality parameters between plasma products that may affect efficacy and safety.

METHODS: Four different plasma products were analyzed to include fresh frozen plasma (FFP), liquid plasma (LP), solvent detergent plasma (SDP), and a spray-dried, solvent detergent-treated plasma (SD-SDP) at multiple time points of storage. Parameters measured included red blood cell, platelet, and white blood cell counts; microparticle phenotypes; thrombin generation; and thrombelastography. These parameters were compared in 10 samples of each product.

RESULTS: SDP and SD-SDP contained the smallest number of residual cells compared with FFP and LP. Platelets were the most common residual cell in all products and were highest in LP. FFP contained the greatest number of residual red blood cells. Total microparticle counts were elevated in LP and FFP compared with SDP and SD-SDP. Cell-derived microparticles in both LP and FFP were mostly platelet in origin. Microparticle counts in SDP and SD-SDP were negligible. Thrombelastography results demonstrated similar thrombin, fibrinogen, and platelet function on Day 28 LP compared with Day 5 thawed FFP. Thrombin generation assays revealed that the total, lag time to, and peak thrombin formation were higher in SDP and SD-SDP compared with FFP and LP. All parameters in FFP and LP products were characterized by a large degree of variability.

CONCLUSION: The differences in cellular, microparticle, and functional hemostatic parameters measured between plasma products have the potential to affect efficacy and safety. Further study is needed to elucidate the potential immune effects of the cellular and microparticle differences noted as well as the clinical implications of altered thrombin generation kinetics in SD products. (*J Trauma Acute Care Surg.* 2015;78:S18–S25. Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.)

KEY WORDS: Plasma quality; thrombin; microparticle; hemostasis; solvent detergent.

The number of plasma transfusions in the United States has been steadily increasing for the past few decades, from less than 2 million units a year in 1979, to 3 million to 4 million units currently transfused per year.¹ For decades, fresh frozen plasma (FFP) was the only product available, but recently, several other types have come onto the market. FFP, plasma frozen at 24 hours (PF-24), and liquid plasma (LP) are single-donor products prepared by separating plasma from whole blood units or by apheresis collection. FFP and PF-24 are frozen at -20°C within 8 hours and 24 hours of collection, respectively, whereas LP is

never frozen but rather stored at 2°C to 6°C for up to 28 days. In contrast, solvent detergent-treated plasma (SDP) products are made from pools of approximately 1,000 FFP units. SDP has been available to patients in Europe for decades, with more than 14 million units transfused. In 2013, the Food and Drug Administration (FDA) licensed Octaplas (Octapharma USA Hoboken, NJ), a solvent detergent-treated product for use in the United States. In addition, a spray-dried SDP product called Resusix, based on the SDP manufactured by Kedrion in Italy is under development by the US Office of Naval Research and the US Department of Defense. The pooling and solvent detergent treatment processes performed to manufacture the Octaplas and Kedrion products are similar.

The FDA allows FFP and PF-24 to be thawed and stored at 1°C to 6°C for 5 days. The AABB, in contrast, allows these thawed plasma products to be stored at 1°C to 6°C for up to 5 days.² A 24-hour period for thawed plasma storage is the standard in many European countries because of concerns over reduced efficacy as a consequence of decreases in coagulation factors, most significantly in factors V and VIII. FDA licensing of Octaplas restricts storage at 2°C to 4°C for 12 hours, with no additional storage time permitted by the AABB.³

Very few studies have compared in vitro plasma product characteristics that may affect quality, at the limit of storage duration. In fact, clinical trials examining the efficacy and safety of different plasma products are also lacking. Recently, a report indicated that LP retained superior hemostatic capacity

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out to 26 days compared with FFP-derived thawed plasma at Day 5.⁴ The authors concluded that their findings were probably caused by higher platelet counts and platelet-derived microparticles in LP. Microparticle content and phenotypes are of particular interest since their cellular origin may potentially have either beneficial or harmful clinical consequences.^{5–23} The objective of this study was to analyze multiple metrics that include residual cell counts, microparticle phenotypes, and functional hemostatic measures of different plasma products. These descriptive data are intended to stimulate further exploration into the clinical relevance of our findings in critically ill patients who require plasma transfusions.

MATERIALS AND METHODS

Ten units of each plasma product tested were analyzed. FFP and LP were purchased from the Blood Centers of the Pacific. Entegion, Inc., provided the SDP and SD-SDP (the SDP used to make SD-SDP was manufactured by Kedrion). All FFP units were type AB, SDP units were from one Type A lot, SD-SDP units were from one Type AB lot, and nine LP units were Type O and one was Type B. FFP and SDP were thawed at 37°C and stored at 2°C to 6°C for 5 days. SD-SDP was reconstituted according to the manufacturer's instructions and stored at 2°C to 6°C for 24 hours. LP was shipped at 4°C to 6°C from San Francisco from Blood Centers of the Pacific to St. Louis; thus, the earliest it could be tested was Day 3. LP was stored for 28 days at 2°C to 6°C. The FFP and LP were not leukocyte reduced after collection.

The sampling times for each of the plasma products were as follows: FFP, Time 0 and Days 3 and 5 after thaw; LP, Days 3, 14, and 28; SDP, Time 0 and Days 3 and 5 after thaw; SD-SDP, Time 0 and 24 hours after reconstitution. FFP stored at 4°C for up to 5 days is commonly called *thawed plasma*; as a result, this term is used throughout the article for this product.

Laboratory Methods

Platelet-Poor Plasma Preparation

Platelet-poor plasma (PPP) was prepared by centrifugation (2,500 G) for 15 minutes and stored at –80°C. For flow cytometry assays, frozen PPP aliquots were thawed for 10 minutes at 37°C and analyzed within 2 hours.

Microparticle-Poor Plasma Preparation

Microparticle-poor plasma (MPP) was prepared for thrombin generation assay testing by centrifuging PPP plasma at 25,000 G for 30 minutes; it was stored at –80°C.^{24–26}

Residual Cell Count Determination

Residual cells were determined by an automated cell counter (Sysmex XN-3000) and by flow cytometry (Becton Dickinson Accuri C6). The automated cell counter was operated in body fluid mode to accurately quantitate rare events. For confirmation, 5 mL of plasma of each product was centrifuged at 3,000 G for 10 minutes, the supernatant removed, and the pellet reconstituted with 500 μ L of phosphate buffer solution. The lower limits of detection were defined as the concentration of cells with coefficient of variation of less than 10% and found

to be 1,000, 5, and 1,000 cells per microliter for red blood cell (RBC), white blood cell (WBC), and platelets, respectively.

Flow cytometry was performed as described by Matijevic et al.⁴ Briefly, 10 μ L of each plasma product was incubated with lineage-specific monoclonal antibodies to identify RBCs (CD235a-APC), platelets (CD41a-PerCP-Cy5.5), and leukocytes (CD45-PE-Cy7) for 15 minutes in the dark. After incubation, samples were diluted with stain buffer up to a total volume of 1 mL. Sample gating was set using the unstained sample. APC-, PerCP-Cy5.5- and PE-Cy7-conjugated isotype control mouse IgG were negative controls. Threshold setting and data acquisition was performed according to software manufacturer recommendations (BD C6 Accuri Cytometer).

Microparticle Phenotyping

Phosphatidylserine-positive (annexin V⁺) microparticles with cellular origins from platelets, RBCs, leukocytes, and endothelial cell-derived microparticle (MP) phenotypes were analyzed in PPP samples using the following antibodies with the BD C6 Accuri flowcytometer: annexin V-FITC, CD41a-PerCP-Cy5.5, CD62P-APC, CD235a-APC, CD47-PerCP-Cy5.5, CD45-PE-Cy7, CD66b-PerCP-Cy5.5, CD3-PE, CD14-APC, CD154-APC, CD105-PE, CD144-PerCP-Cy5.5, CD62E-APC, CD106-APC, CD142-PE, and CD35-PE. BD TruCOUNT tubes quantified absolute MP counts within different plasma products. Small Bead Calibration Kits (Bangs Laboratories) were used to measure microparticles within the constant region of 0.2 μ m to 1 μ m (Supplemental Digital Content 1, <http://links.lww.com/TA/A554>). Positive control acquisition was based on full stains minus one, and the compensation was set against antibody capture beads with single and tandem fluorochromes. The potential clinical relevance of each of the microparticles measured is listed in Supplemental Digital Content 2, <http://links.lww.com/TA/A554>. Ten microliters of PPP from each plasma product was incubated with 2 μ L of lineage-specific monoclonal antibodies with or without 2 μ L of annexin V (in the presence of 2.5-mM CaCl₂) for 30 minutes at room temperature in the dark. After incubation, samples were diluted with stain buffer or annexin V binding buffer up to a total volume of 300 μ L. Data acquisition was performed as suggested by the software manufacturer (BD C6 Accuri).

Coagulation Parameters

Thrombelastography (TEG) testing was performed with kaolin activation of 340 μ L of plasma in standard TEG cups with 20 μ L of 0.2-M CaCl₂ in FFP and LP samples only. The absence of phospholipids and platelets in SDP and SD-SDP samples does not allow for clot formation with standard kaolin-activated TEG testing. Clot reaction time (*R* time), ΔR time (*R* time – split point time), clot formation time (*K* time), propagation time (α angle), maximum clot amplitude (MA), and shear elastic modulus (*G* value) were determined. The ΔR time is thought to reflect thrombin generation more accurately than the *R* time;^{27,28} however, this concept has not been well studied; thus, both values are reported.

Thrombin production was determined by a thrombin generation assay, using the Calibrated Automated Thrombogram (CAT) (Thrombinoscope).²⁹ Thrombin generation reaction was triggered by PPP-low reagent that produces 1-pM TF final

concentration in a sample. Each sample was analyzed in triplicate, and the data were averaged.

Statistical Analysis

Continuous demographic and clinical variables are reported as mean (SD) if parametric or median with interquartile range (IQR) if nonparametric and categorical variables as counts and percentages. Continuous data were assessed with the Student's *t* test or Wilcoxon rank-sum test, as appropriate. Data for more than two groups with single time measurement were assessed with one-way analysis of variance (ANOVA). Data for more than two groups of data with repeated measures were assessed with two-way ANOVA with Bonferroni postadjustment as appropriate. Comparisons were considered significant if the two-sided *p* value was <0.05. Analyses were performed using SAS and GraphPad Prism.

RESULTS

Residual Cell Counts

In general, platelets were the most common residual cell in the plasma products tested, with more detected in LP than SD or SD-SDP by both counting methods (Table 1). Residual RBCs were low in all products and were significantly increased in FFP compared with SD-SDP by the Sysmex method only. WBC counts were low in all plasma products and marginally higher in LP and SD-SDP than in SD by Sysmex counting and higher in LP compared with FFP and SD-SDP by flow cytometry.

Microparticle Count and Phenotypes

Total annexin V⁺ and specific MP phenotype absolute counts are reported in SDC 3, <http://links.lww.com/TA/A554>.

TABLE 1. Residual Cell Counts According to Sysmex Cell Counter and Flow Cytometry for Each Plasma Product at Day 0 for FFP, SDP, and SD-SDP, and Day 3 for LP

Sysmex			
	Median (IQR), cells/ μ L		
	RBC	WBC	Platelets
FFP	100 (0.0–125)*	0.5 (0.0–25.3)	800 (450–1,450)**
LP	0 (0.0–100)	4.5 (0.0–12.5)†	2,500 (1,800–3,950)†‡
SD	0 (0.0–0.0)	0 (0.0–1.3)†¶	0 (0.0–0.0)**†
SD-SDP	0 (0.0–0.0)*	5 (4.0–10.0)¶	200 (175–225)‡
Flow cytometry			
	Median (IQR), cells/ μ L		
	RBC	WBC	Platelets
FFP	233.5 (103.8–415.8)	0.0 (0.0–9.5)	959.0 (784.8–1,514.0)*
LP	203.0 (145.3–316.8)	23.5 (3.0–46.8)‡	8,454 (4,767–22,424)†‡
SD	125.5 (83.0–210.0)	7.0 (3.0–9.8)	700.5 (303.8–782.0)†
SD-SDP	186.5 (172.8–225.8)	1 (0.0–3.0)‡	294 (269.5–422.5)*†‡

*Significant between FFP versus SD-SDP.

**Significant between FFP versus SDP.

†Significant between LP versus SD.

‡Significant between LP versus SD-SDP.

¶Significant between SD versus SD-SDP.

||Significant between FFP versus LP.

Values presented as median with IQR. For all significant comparison, *p* ≤ 0.05.

Concentrations for each plasma product tested for annexin V⁺ microparticles (total, platelet-derived [CD41a], and RBC-derived [CD235a and CD47]) over multiple time points are displayed in Figure 1. The number of microparticles from any cellular source is negligible in SDP and SD-SDP. After adjustment for multiple comparisons, platelet-derived microparticles are the most common subtype in LP and thawed FFP. CD235a⁺ microparticles increased over time in thawed FFP, from Day 0 to Day 5. For Day 3 LP, CD41a-positive microparticle counts were higher than for CD235a and CD47 by ANOVA (*p* < 0.0001). For Day 0 FFP, CD41a-positive microparticle counts were higher than for CD235a and CD47 by ANOVA (*p* < 0.005). Measurements of leukocyte-derived (CD45⁺) microparticles in FFP and LP were numerically increased but not significantly, compared with SDP and SD-SDP (Supplemental Digital Content 4, <http://links.lww.com/TA/A554>). The number of unspecified microparticles (total microparticles – sum of all analyzed) were numerically higher in FFP and LP compared with SDP and SD-SDP (Supplemental Digital Content 3, <http://links.lww.com/TA/A554>).

TEG Analysis

Kaolin activation did not cause clot formation in SDP and SD-SDP samples; thus, these data are not shown. After adjustment for multiple comparisons, as storage duration of thawed plasma increased, the time to initial fibrin formation (increased *R* time and ΔR time) and fibrinogen function (*K* time and α angle) did not change significantly (Table 2). Clot strength (MA and *G* values) also did not change over time in thawed FFP, but the baseline (Day 0) measurement was significantly lower than Day 3 LP, which contained increased platelets compared with Day 0 thawed FFP. All TEG parameters were similar for LP at Day 28 compared with Day 5 thawed FFP. There was no measurable fibrinolysis in either thawed FFP or LP at any time point.

Thrombin Generation

Figure 2 displays thrombin generation assay results over time, and Figure 3 shows the individual sample curves on the first day of analysis, demonstrating the variability found within FFP and LP products compared with SDP and SD-SDP, which were obtained from the same batch of pooled plasma. After adjustment for multiple comparisons, there was no significant difference for thrombin generation lag time between products compared, except for Day 28 LP compared with Day 5 SDP (Fig. 2A). Endogenous thrombin potential (ETP), a measure of total thrombin formed, was highest in SDP and thawed FFP on Day 0 and decreased over time in thawed FFP (Fig. 2B). ETP values were increased for Day 5 SDP compared with both Day 5 thawed FFP and Day 28 LP, whereas the ETP values were similar between Day 5 thawed FFP and Day 28 LP. Thrombin peak values were significantly higher for Day 0 SDP than for SD-SDP. Thrombin peak values were also significantly higher for Day 5 SDP compared with Day 5 thawed FFP and Day 28 LP. Day 5 thawed FFP and Day 28 LP had similar ETP values. Thrombin peak values decreased over time significantly in thawed FFP and SDP (Fig. 2C).

To assess if the microparticles in each product tested affected thrombin generation, PPP samples were compared with MPP samples (Table 3). After adjustment for multiple

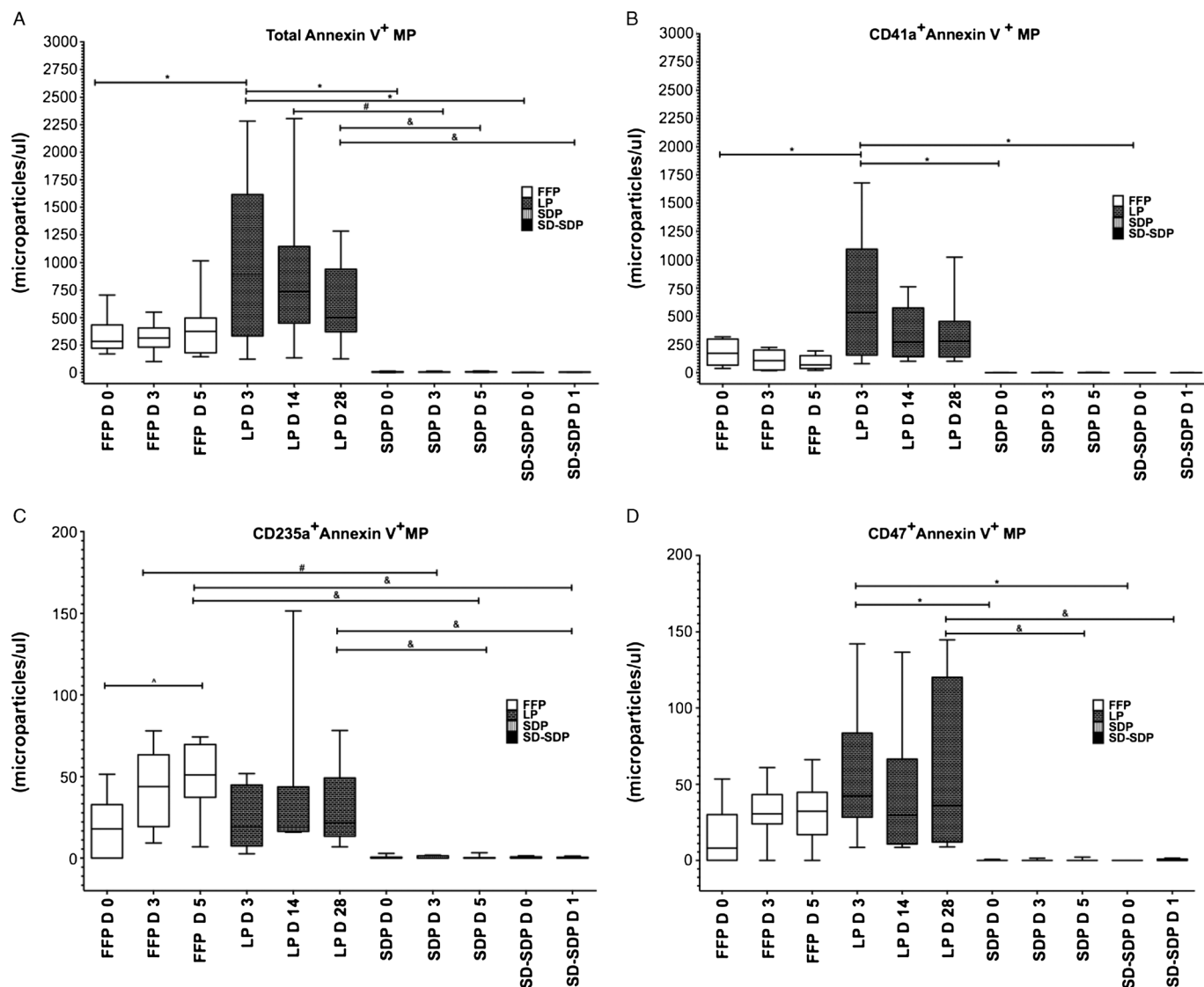


Figure 1. Microparticle concentrations in each plasma product at multiple storage duration times. *FFP D0 versus LP D3 versus SDP D0 versus SD-SDP D0. #FFP D3 versus LP D14 versus SDP D3. &FFP D5 versus LP D28 versus SDP D5 versus SD-SDP D1. ^Within products. Graphs depicted as median values with IQR. For all significant comparison, $p \leq 0.05$.

comparisons, there was increased thrombin generation in FFP and LP samples at all time points in PPP versus MPP samples, as expected. There was no difference in PPP versus MPP in

both of solvent detergent-washed plasma, which was also expected because of very low counts of microparticles detected in both of these plasma products.

TABLE 2. TEG Parameters for Plasma Products at Multiple Time Points of Storage Duration

TEG Parameters (Reference Value)	Thawed FFP Day 0	Thawed FFP Day 3	Thawed FFP Day 5	LP Day 3	LP Day 14	LP Day 28
R time (5–10 min)	7.9 ± 1.2	11.9 ± 2.6	12.0 ± 1.6	12.0 ± 1.4	11.4 ± 1.0	13.3 ± 1.5
ΔR time (0.7–1.1 min)	0.6 ± 0.1	0.4 ± 0.1	1.0 ± 0.3	0.9 ± 0.2	1.0 ± 0.2	1.9 ± 0.8
K time (1–3 min)	2.1 ± 0.5	2.2 ± 0.7	2.7 ± 0.7	2.5 ± 0.6	2.9 ± 0.7	3.4 ± 0.9
α angle (53–72 degrees)	67.6 ± 1.5	66.6 ± 5.0	59.3 ± 5.2	61.1 ± 2.3	58.5 ± 4.2	50.3 ± 6.2
MA (50–70 mm)	27.4 ± 1.2*	28.3 ± 0.9	28.5 ± 0.6	41.9 ± 3.7***	32.4 ± 2.8	28 ± 2.3**
G value (4.5–11 Kd/sc)	1.9 ± 0.1*	2.0 ± 0.1	2.0 ± 0.1	3.9 ± 0.5***†	2.5 ± 0.3†	2 ± 0.2**

*Significant between FFP D0 versus LP D3.

**Significant between LP D3 versus LP D28.

†Significant between LP D3 versus LP D14.

Values presented as mean ± SEM. For all significant comparison, $p \leq 0.05$.

DISCUSSION

This is the first study to compare multiple characteristic measures of plasma to include residual cell counts, microparticle

counts according to phenotype, and hemostatic parameters between single-donor, pooled, and spray-dried plasma products. Surprisingly, plasma quality measures in the United States have not been previously established. The FDA does not require specific quality parameters or criteria for plasma but rather licenses the plasma processing method. Conversely, the European Union has established plasma criteria including pH (6.5–7.6), osmolality (minimum of 240 mOsm/kg), total protein content (minimum of 45 g/L), absence of irregular RBC antibodies, hepatitis A virus antibody levels, and coagulation factor concentration ranges. The coagulation factor criteria include factors VIII, V, XI, (>0.5 IU/mL), protein C (>0.7 IU/mL), and α -2 antiplasmin (>0.2 U/mL). These parameters provide an initial framework to establish a more thorough panel of quality measures that are also clinically relevant.

Another emerging measure of plasma efficacy may also be its ability to protect or restore endothelial function during acute hemorrhage. Future quality metrics should assess plasma's ability to prevent or treat the endotheliopathy of trauma,^{30,31} a condition characterized by endothelial dysfunction, barrier integrity compromise, deregulated coagulation, and overt inflammation. Previous studies comparing FFP with SDP and SP-SDP revealed similar efficacy in restoring the endothelium and reducing inflammation within the pulmonary endothelium³² both in vitro and in animal models.

The implementation of damage-control and hemostatic resuscitation principles at many large tertiary care centers has greatly increased the need for immediately available plasma. Simultaneously, physicians are placing greater emphasis on optimization of risk-benefit profiles and looking for products with enhanced safety profiles that do not compromise efficacy. Well-designed studies that examine efficacy and safety of plasma products available have not been performed because hospitals shift to immediately available plasma products for severe hemorrhage. Data from the Department of Health and Human Services indicate that the vast majority of all plasma being transfused in the United States in 2008 was either FFP (54%) or PF-24 (27%) and that thawed plasma use was negligible. Just 3 years later, the use of FFP and PF-24 had declined to 37% and 33%, respectively, and thawed plasma increased to 30% in the absence of any reported safety or efficacy data.³³

Despite a 10-fold higher platelet count in the LP samples tested by Matijevic et al.,⁴ our data are consistent with their recently published results comparing FFP-derived thawed plasma to LP. Both studies found that there was no difference between Day 5 thawed plasma and Day 28 LP for clot firmness (MA and *G* value), and thrombin generation parameters. An initial evaluation of endothelial function also reported that there were similar in vitro endothelial protective effects when

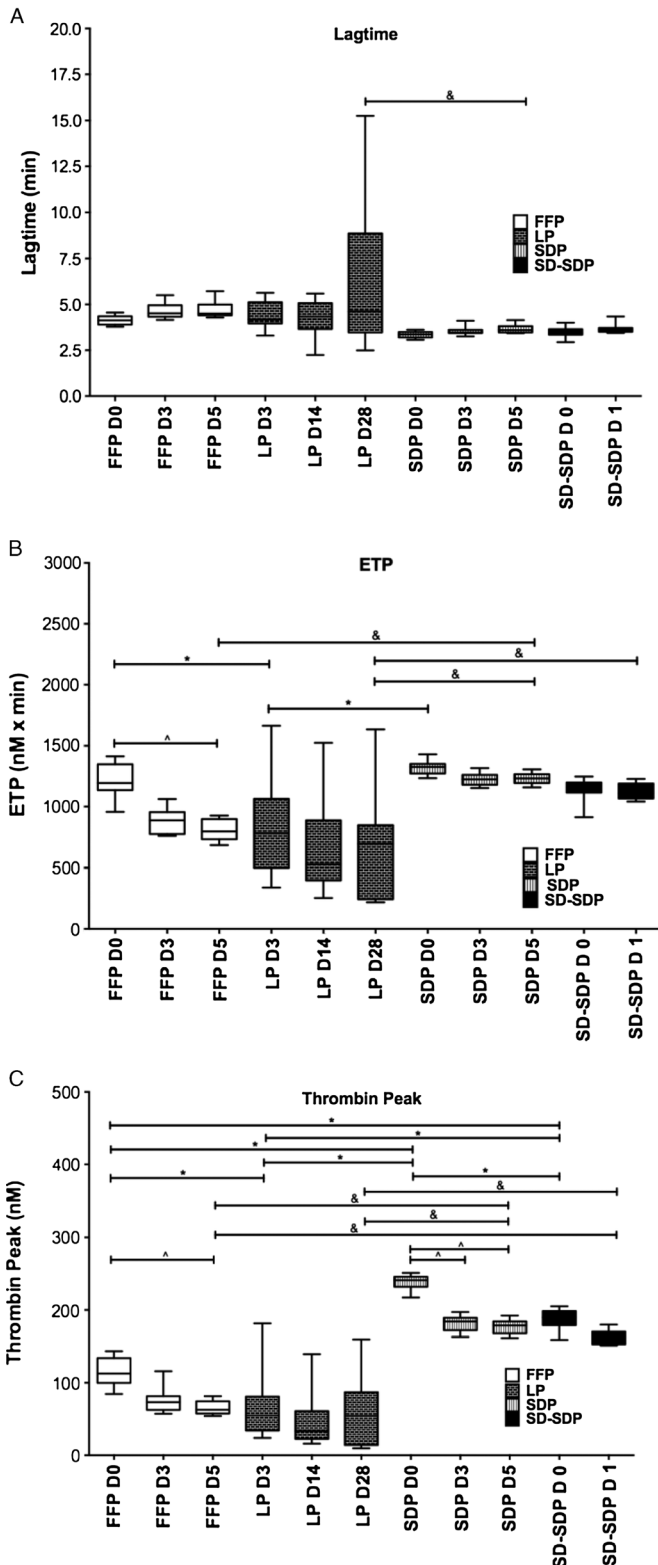


Figure 2. Thrombin generation assay results for plasma products at multiple storage duration times and individual products for each plasma product at initial storage time tested. *FFP D0 versus LP D3 versus SDP D0 versus SD-SDP D0. #FFP D3 versus LP D14 versus SDP D3. &FFP D5 versus LP D28 versus SDP D5 versus SD-SDP D1. ^Within products. Graphs depicted as median values with IQR. For all significant comparison, $p \leq 0.05$.

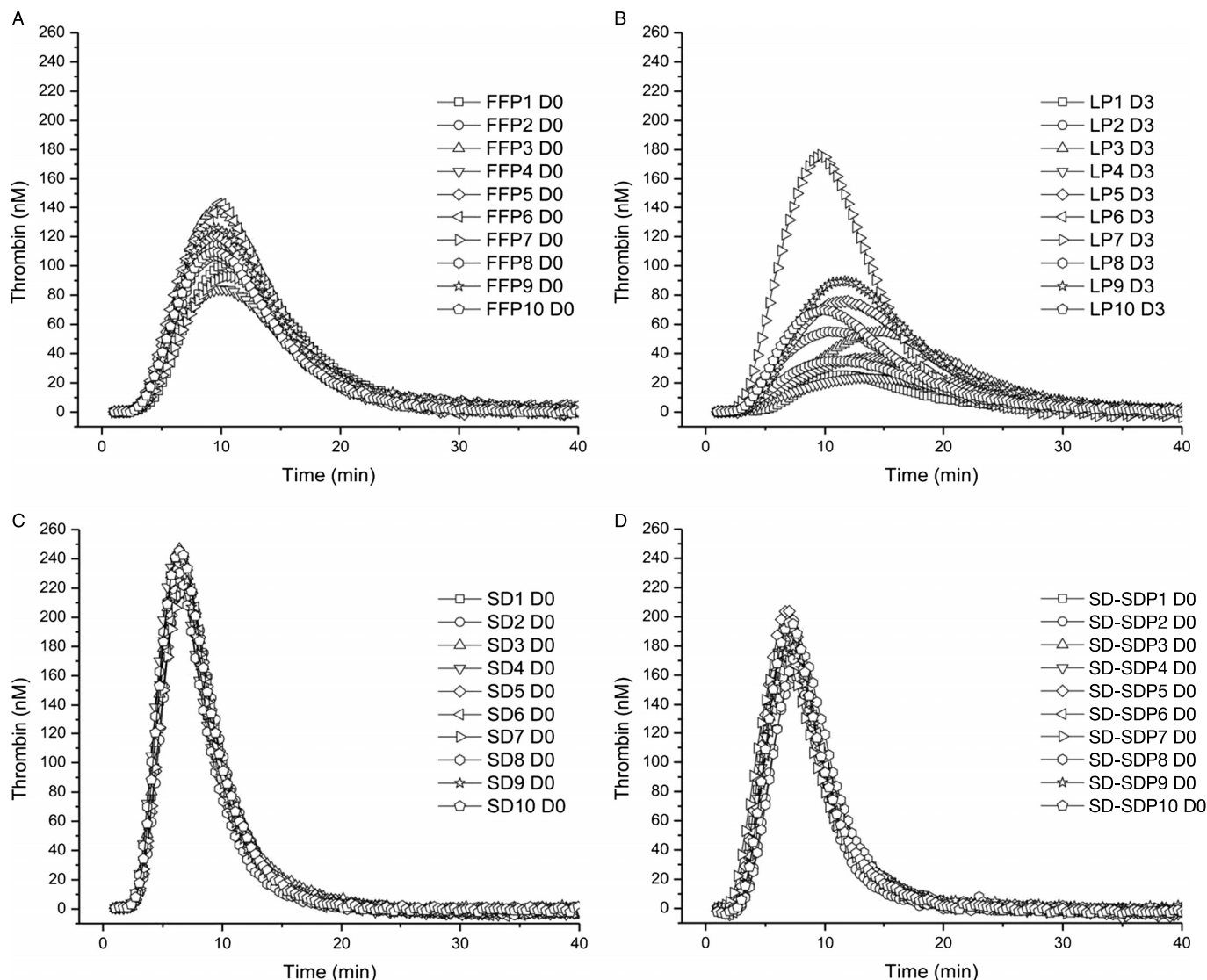


Figure 3. Individual thrombin generation curves for each plasma product at initial storage time tested.

Day 5 thawed FFP was compared with Day 28 LP.³⁴ If similar hemostatic and endothelial efficacy of Day 28 LP compared with Day 5 thawed FFP is demonstrated in clinical trials, this could dramatically increase the feasibility of providing immediately available plasma while also reducing the logistic hurdles of requiring frozen plasma to be thawed. There could be an increased risk of plasma wastage since the shelf-life of LP is 28 days versus 1 year for FFP however. The cost-effectiveness of LP versus thawed FP requires further study.

Residual cell and microparticle counts are a potential clinically relevant quality metric for plasma because of their hypothesized role in the immunosuppression underlying transfusion-related immune modulation. Notable findings in this study included increased CD41, CD235a, CD47, and CD45 microparticles in thawed FFP and LP, since they are associated with immune suppression and prothrombotic states (Supplemental Digital Content 2, <http://links.lww.com/TA/A554>). This may account for the adverse effects that have been associated

TABLE 3. Differences in Thrombin Peak Values Between PPP and MPP Samples

Thrombin Peak, nM	Thrombin Peak, nM		
	PPP Median (IQR)	MPP Median (IQR)	<i>p</i>
FFP D0	112.7 (99.8–133.9)	90.7 (81.7–96.2)	0.002
FFP D3	73.2 (62.6–81.5)	58.0 (47.7–70.9)	0.0045
FFP D5	62.7 (57.6–74.5)	50.8 (44.7–60.3)	0.0039
LP D3	54.9 (34.1–80.9)	44.2 (18.7–60.0)	0.002
LP D14	33.4 (22.5–60.9)	20.9 (15.6–54.2)	0.0078
LP D28	54.3 (14.1–87.4)	32.1 (9.9–43.1)	0.0078
SD D0	241.1 (231.9–245.7)	242.1 (226.6–250.2)	0.959
SD D3	184.2 (172.2–189.3)	182.7 (170.5–197.7)	0.7394
SD D5	178.9 (167.9–184.2)	176.3 (169.6–181.6)	0.7394
SD-SDP D0	189.7 (179.1–198.6)	183.1 (174.2–203.3)	0.642
SD-SDP D1	163.4 (152.7–170.3)	171.4 (155.5–178.6)	0.393

with FFP in the literature. Conversely, certain microparticles may play an important role in hemostasis, so their presence could contribute to the procoagulant activity of these products. However, it is curious that total thrombin formation was higher in SDP and SD-SDP, which are practically devoid of microparticles. The clinical impact of the transfusion of plasma with or without the microparticle phenotypes described is unknown and may be different according to the nature of the patient's critical illness. Future clinical studies that analyze the clinical effects of microparticle content within plasma products are essential since there are wide differences between products and there are potential adverse or beneficial effects.

Our results indicated wide donor variability in single-donor plasma products (FFP and LP) in all parameters measured. Most striking is the variability in thrombin generation potential by the 10 different donors of FFP and LP tested. The relevance for clinical efficacy and safety of donor and product variability in residual cell counts, microparticle counts, or coagulation parameters are unknown and require further analysis. The relative absence of variation in the two pooled plasma products analyzed is similarly expected from a single lot. Further studies comparing multiple lots of pooled plasma products are needed.

Previously reported ranges for residual cells in plasma products are between approximately 0 to 950 cells per microliter for WBC, 0 to 11,400 cells per microliter for RBC and 0 to 38,000 cells per microliter for platelets.³⁵ Our results varied significantly depending on the method used. The cell count analyzer uses light scatter and fluorescent stains to differentiate a broad spectrum of cell types present in blood. The instrument uses fluorescent dyes that stain nucleic acid to determine viable cells, which detects cells with a variability of less than 10% in standard patient samples. While the method is accurate and sensitive in whole blood specimens, the sensitivity decreases in plasma samples. Our samples were centrifuged to concentrate residual cells, which may further contribute to inaccuracies. Flow cytometric methods to count cells in plasma products may also be inaccurate since cell counts can be overestimated because of carryover of sample material or if cell fragments from nonviable cells are counted. Regardless of the method used, residual platelets were found in both FFP and LP products and a small residual amount of RBCs in FFP.

Comparative microparticle phenotype counts for plasma products have not been previously described. Consistent with the number of residual cells counted, we found that the majority of microparticles in FFP and LP were platelet derived, followed by RBC, leukocyte, and endothelial cell in origin, whereas SD-SDP and SD plasma contained significantly fewer microparticles. A large number of microparticle phenotypes were not captured by our panel, as evidenced by a significant number of uncategorized microparticles.

While it has been standard practice to measure specific factors or proteins between products as a reflection of efficacy, we measured global and functional measures of hemostasis with the assumption that they more accurately reflect in vivo function. The increased platelet content in LP accounts for the increased maximal amplitude and *G* value results compared with FFP. Thrombin generation assay kinetics differed between single-donor and SD-treated pooled products. Total thrombin

formation was higher in SDP and SD-SDP compared with FFP and LP products. The reduced time to peak and higher peak thrombin generation in the SD-treated pooled products suggest that more rapid and stronger thrombin generation is followed by a precipitous decline compared with single-donor plasma products. The clinical relevance of this difference is unknown. It is theoretically possible that an elevated thrombin generation peak could promote thrombotic events, or conversely, the effect of the rapid reduction of thrombin generation could reduce this risk. Clinical trials comparing these plasma products are required to further elucidate these findings. One small randomized controlled trial in liver transplant patients with severe bleeding indicated that with TEG-directed plasma transfusion, less SDP than FFP was required to achieve the same hemostatic effects.³⁶

Our study was limited by the inability to assess LP on Day 0 because of shipping time. In addition, we were unable to match ABO groups between plasma products. This may have influenced coagulation testing, as Group A donors are known to have increased hemostatic activity compared with Group O donors. Future studies should include leukocyte stimulation tests with and without cells and microparticles to obtain in vitro data on the potential immune effects.

CONCLUSION

Cellular, microparticle, and hemostatic metrics differ between plasma products. Day 28 LP is similar in hemostatic efficacy and microparticle load by in vitro measures compared with FFP-derived thawed plasma. SD-treated pooled products have reduced residual cell and microparticle counts and altered thrombin generation kinetics compared with FFP and LP. The clinical relevance of the differences noted in our study need to be prospectively evaluated in critically ill populations.

AUTHORSHIP

P.C.S. and E.F. are guarantors of the integrity of the entire study. P.C.F., E.F., and O.G. provided the study design. E.F. and O.G. performed the data acquisition. All authors performed the analysis and interpretation of data. EF, HFP, and JKA performed the statistical analysis. All authors participated in the manuscript preparation. All authors participated in the manuscript editing. All authors also participated in the manuscript revision/review and final version approval.

DISCLOSURE

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